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The Responses of Ecologically Dissimilar Populations of the Water Snake, *Natrix sipedon sipedon* to Surface Extracts of Prey Species, With Observations on Feeding and Defense Behaviors

Doris Gove
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To the Graduate Council:

I am submitting herewith a thesis written by Doris Gove entitled "The Responses of Ecologically Dissimilar Populations of the Water Snake, *Natrix sipedon sipedon* to Surface Extracts of Prey Species, With Observations on Feeding and Defense Behaviors." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

J. C. Howell, Major Professor

We have read this thesis and recommend its acceptance:

G. M. Burghardt, R. R. Schmoller

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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November 21, 1971

To the Graduate Council:

I am submitting herewith a thesis written by Doris Gove entitled "The Responses of Ecologically Dissimilar Populations of the Water Snake, Natrix sipedon sipedon to Surface Extracts of Prey Species, With Observations on Feeding and Defense Behaviors." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Zoology.

J. C. Howell
Major Professor

We have read this thesis
and recommend its acceptance:

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Accepted for the Council:

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Graduate Studies and Research

THE RESPONSES OF ECOLOGICALLY DISSIMILAR POPULATIONS OF THE WATER SNAKE, NATRIX SIPEDON SIPEDON TO SURFACE EXTRACTS OF PREY SPECIES, WITH OBSERVATIONS ON FEEDING AND DEFENSE BEHAVIORS

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Presented to

the Graduate Council of

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Doris Gove

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Mr. J. B. Jordan drew Figure 1.

My brother, Dr. Norwood Gove, programmed my graphs in the computer.

Mr. Robert Stiles helped me catch and identify fish from the Little River.

The National Park Service.

ABSTRACT

Observations of defensive and feeding behavior of Natrix sipedon were made in the field and in the lab. Crypticity, mimicry of poisonous snakes, striking, production of cloacal secretion and tail autotomy were defensive behaviors discussed. Feeding behaviors discussed were hunting, catching prey and tongue-flicking.

Experiments were performed on the responses of snakes from ecologically dissimilar populations of N. sipedon to surface extracts of local prey species. The populations were: (1) a laboratory-reared litter of ten one-year-old snakes; (2) six wild-caught snakes from Sterchi's fish hatchery in north Knoxville, Tennessee; and (3) six wild-caught and eight newborn snakes (a litter) from the Tremont area of the Great Smoky Mountains National Park. Extracts were made of prey species caught in both areas or obtained in the laboratory. Response scores were combined measure of tongue-flicks and attack latency.

Group one snakes responded significantly more to goldfish (C. auratus), than to any other extract. This species had been their food for one year. Group 2 also responded more to C. auratus than to any other fish extract. The goldfish was abundant at Sterchi's. However, Group 3

responded significantly more to extracts of fish that were caught near Tremont than to C. auratus. That snakes discriminated between different genera of prey was shown. The conclusion was made that wild-caught snakes respond more often to extracts of sympatric prey, presumably because these snakes have had experience eating this prey. Young of the Tremont snakes responded more to extracts of sympatric prey than to C. auratus, but the difference was not significant. This technique can be used, in conjunction with other investigations, to assess ecological relationships in an area.

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CHAPTER 1

INTRODUCTION

I. DESCRIPTION OF THE SPECIES

Natrix sipedon sipedon /Linne_7, the northern water snake (Colubridae), is a common snake ranging from the Gulf of Mexico to southern Ontario and northern Maine, and west to eastern Colorado (Conant, 1958). It lives on creeks, rivers, bogs, ponds, lakes - practically any permanent body of water, and it uses water for refuge, food gathering, and in some cases, hibernation (Brown, 1940). Reproduction is ovoviviparous, with nine to 70 young born in late summer or fall (Wright & Wright, 1957).

This snake has been described by Littleford (1946) as "uninteresting and difficult to handle," compared with other snakes, and this attitude may explain why, in spite of its abundance, the northern water snake has not received much attention since Brown's dissertation (1940) on its life history and habits. The snakes used for my work, however, were both interesting and easy to handle.

The food of the northern water snake has been described by many authors who examined stomach contents, either by cutting open snakes or by inducing vomiting. Foods listed,

in general categories, are fish (predominant, according to all authors), frogs, tadpoles, salamanders, crayfish, insects and mammals. Table I documents the main food genera found. Insect remains found in stomachs may have been eaten by sipedon's prey rather than the snakes themselves (Neill and Allen, 1956). Brown (1940) found no evidence in his extensive studies of food habits that water snakes ate insects, mammals or birds. Mammals and crayfish were only rarely found in stomachs; Brown stated that food-deprived snakes will not eat a crayfish even when it is covered with fish odor. Toads were mentioned as a snake food by Brown, who only found a few instances of toads in stomachs. No author mentioned turtles as snake food, but sipedon at the Knoxville Zoo has eaten newly hatched box turtles (Terrapene carolinensis)(Larry Brown, Reptile Curator, pers. comm.). Brown (1940) analyzed prey animals found in sipedon stomachs in New York State, and found that 80% of the food was fish, with minnows (Notropis), darters (Etheostoma), cottids (Cottus), and suckers (Catostoma) predominating.

Senses of snakes related to feeding

Work has been done on the sensory capacities of many "natricine" snakes. "Natricinae" is a subfamily of Colubridae which is recognized by some herpetologists, such as Conant

TABLE I. Food genera most commonly found in stomachs of *Natrix sipedon*

Genus	Source
FISH	
Notropis	Raney & Roecker, 1947; Brown, 1940; Conant, 1938.
Campostoma	Raney & Roecker, 1949; Huheey & Stupka, 1967; Brown, 1940.
Nocomis	King, 1939; Huheey & Stupka, 1967; Brown, 1940.
Cottus	King, 1939; Huheey & Stupka, 1967; Brown, 1940.
Carassius	Fraker, 1970; Brown, 1940.
Hybopsis	Huheey & Stupka, 1967; Brown, 1940.
Etheostoma	Brown, 1940.
Osmerus	Dix, 1968.
Salvelinus	Brown, 1940; King, 1967.
AMPHIBIANS	
Rana	King, 1939; Fraker, 1970; Huheey & Stupka, 1967; Brown, 1940; Evans, 1942.
Desmognathus	King, 1939; Huheey & Stupka, 1967; Brown, 1940.
Bufo	Brown, 1940.
OTHER	
Crayfish	Fraker, 1970.
Mammals	Raney & Roecker, 1947.
Insects	Huheey & Stupka, 1967.

(1957) and Goin and Goin (1962). It includes the genera Natrix, Thamnophis and Storeria, and is based on the many morphological relationships among them. Most of the sensory work had been done on other members of the subfamily than Natrix, but may be generalized.

Visual Stimuli. Snakes respond to movement more than to form (Weidmann, 1932, quoted by Burghardt, 1970). A visual stimulus of movement may elicit interest, as evidenced by increased tongue-flicks and motor activity, but the visual cue alone is not sufficient to elicit attack in newborn Thamnophis (Burghardt, 1960).

Chemical Stimuli. Wilde (1938) showed, through surgical cutting of nerve pathways, that the chemical stimuli which elicited an attack response to prey extracts in Thamnophis sirtalis were received by the tongue and Jacobson's organ, not olfaction. Burghardt and Hess (1968), presenting extracts on cotton swabs, compared the responses of blind and/or anosmic Thamnophis radix to normals. They found no reduction of response with either treatment or both together.

Former work on snake food preference

Snakes respond more to chemical surface extracts from

species-characteristic prey than to prey that they do not eat. The response can be measured by the number of tongue-flicks per unit time in the presence of the extract, or by the latency and frequency of attacks (Wilde, 1938; Burghardt, 1967; Loop, 1970b).

Natricine snakes and many others are born with an innate preference for a category of food, as inferred from different responses to extracts (Burghardt, 1967; 1969). This preference may help to prevent species competition of related snake species living in the same area (Burghardt, 1968). The innate responses to particular extracts by newborn snakes of the same species from different geographical areas may be different and probably correspond to the types of prey that are available (Dix, 1968; Burghardt, 1970a). The snake populations sampled by Burghardt had a high degree of genetic isolation (coming from Illinois, Iowa and Wisconsin), and presumably their differences in response were due to natural selection mediated by the prey species available.

It has also been shown that early feeding experience affects the response of snakes to water extracts of prey (Fuchs and Burghardt, 1971). In their study, a litter of Thamnophis sirtalis was divided into two groups and fed two different prey items (redworms and guppies). Before feeding

and during the feeding schedules, they were tested with surface extracts. The snakes' responses to the extracts of the food they were eating increased, while extracts of the food not offered declined in effectiveness. The snakes were found to be able to discriminate between two species of annelid worm which had initially had similar releasing values. This indicates that snakes of the same species may develop marked differences in chemical preferences because of the differences in prey availability. The innate prey extract response profiles thus may be altered by feeding experience.

III. GENERAL PROCEDURES

I extensively observed watersnakes in the laboratory and field to familiarize myself with their behavior, after which I chose one behavior, food selection, for quantitative experimentation.

Observations

Wild and captive snakes were observed. The wild snakes used lived in two areas of eastern Tennessee. One area, Tremont, is in the Great Smoky Mountains National Park (GSMNP) on the Middle Prong of the Little River, at an altitude of 412 meters. Near the river are two 20-meter diameter settling ponds for a small sewage treatment plant. The river has riffles, pools and rapids, with many large boulders. The water is very clear except after a heavy rain. Around the ponds and in some places near the river banks, tall grass dominates, but elsewhere the vegetation is non-climax mixed hardwoods. Most of the ground area is shady. Water snakes in this area have an abundance of fish, frogs, toads and salamanders to eat, and may be preyed upon themselves by small mammals, raptorial birds, other snakes, and perhaps large fish. Like other Park animals, snakes are protected from human attackers, though fishermen, believing the snakes are competing for the best fish, may break the law.

The other area, Mr. James Sterchi's Fish Hatchery, is in northern Knoxville, and is an open field with over 100 small spawning ponds about 15 meter diameter. The altitude is 330 meters. A small shallow creek runs through the field. Water depth ranges from seven cm to 50 cm; width is from one meter to almost two meters. The creek has a gravel and mud bottom. The water is generally clear but often stirred up by horses. Tall weeds and brush line the creek, but the areas around the ponds are mowed or grazed by sheep and horses. The one shade tree in the field was cut down in July. At least three species of frogs, one toad, and a few fish species are available for food. Snakes and other wild animals are shot on sight by Mr. Sterchi and his employees. However, he was otherwise cooperative, though somewhat bewildered by my endeavors.

Snakes in both of these areas were observed during the summer of 1971. Observational notes were also made on laboratory snakes and snakes in outside enclosures.

Experiments

Food preference experiments were carried out using skin extracts of prey species, which were collected from the same areas where the snakes lived. An attempt was made to see if captured snakes from different areas responded differently to different species of fish and amphibians.

CHAPTER II

OBSERVATIONAL WORK

I. AREAS OF OBSERVATION

Water snakes were observed in three areas: (1) the Tremont collection area, (2) the Knoxville collection area: Sterchi's bait farm, and (3) enclosures with sheet metal walls in a fenced area of open woods near the UT Hospital. Many hours at Sterchi's were unproductive; snakes simply were not seen, though they could be collected by trapping and searching under stones. It is possible that they are more sensitive to the presence of humans than those at Tremont, since for many years snakes have been shot on sight by Mr. Sterchi and his employees. Consequently, the bulk of the observations of wild snakes occurred at Tremont.

II. SCHEDULE AND METHOD OF OBSERVATION

The first Park observations were in May, 1971, and they continued through August, 1971. Observation periods lasted three or four hours and occurred two or three times a week. Binoculars were used for observing. In May and early June, snakes were seen on and in the settling pond for the Tremont Sewage Treatment Plant. In June, the ponds became

covered with duckweed and algae. The snakes left the ponds, possibly because of the weeds, and could only be seen on the river.

At the UT Hospital, one-year-old snakes that were retired from testing were installed in the enclosures. Live food was supplied for them in two-foot diameter ponds. These ponds were one foot deep and lined with heavy polyethylene.

III. SNAKES' AWARENESS OF OBSERVER

Some speculations can be made on the snakes' awareness of the observer. My observations indicated that the snakes were not visually aware of a still human form. However, they seemed to be aware of me by chemical senses if within eight feet. Snakes observed close to me tongue-flicked more than those twenty or more feet away, and often tongue-flicks were directed toward me. This awareness was not a sufficient stimulus for escape behavior.

Small movements such as a light shifting of binoculars or a finger motion, elicited increased tongue-flicks. A snake would stop locomoting and tongue-flick five to ten times then remain motionless, and then start tongue-flicking again. After about five minutes, it would start moving again. It seemed that the distance from the snakes' head to

the ground on an open substrate (without grass) is correlated with its degree of attentiveness. This was not tested, but I observed that when I startled a moving snake, it subsequently moved with its head held higher. Also, the periods of tongue-flicking became longer.

A more intense visual stimulus, such as moving my foot, dropping a pencil, or lifting binoculars, usually elicited rapid escape into water. These stimuli seemed equally effective if the snake was close (within eight feet) or far (more than twenty feet away).

IV. DEFENSIVE BEHAVIOR

Cryptic defense

A water snake's best defense, at least against humans, is not being seen in the first place. Since man is the main attacker of water snakes (Brown, 1940; Conant, 1958), this crypticity is significant in the snake's life. Their color and pattern help snakes to be cryptic, and certain behaviors increase this quality.

Crawling. Water snakes were observed in thick grass, on rocks, on pebbles, and in the water. The type of locomotion and the substrate and conditions were recorded.

Terrestrial locomotion is usually slow if the snake

is not alarmed. On all substrates, all parts of the snake's body follow the same path along the ground. Since the dorsal patterns are indistinct when dry, the snake is thus not seen to move in relation to its surroundings. There is no motion to catch the observer's eye. The curves of the snake's body often follow natural curves, such as the edges of rocks or logs. This also makes it harder to see.

Waving. In thick grass, water snakes move often with their heads from three to 15 cm from the ground, and the raised part of the body moves in a jerky or wavy motion. The rest of the body moves through the grass in the manner described above. I feel that this behavior in the water snakes serves a cryptic function, since a still object in waving grass would be more conspicuous than a waving object. It may also serve to increase parallax vision. However, I never observed this behavior on a flat substrate or on one without grass. The snakes born in the lab did not exhibit this waving behavior in their tanks, but when they were moved to pens containing grass and tall weeds, they performed it immediately. I have observed similar behavior in African Chameleons (Chameleon bitaeniatus and C. jacksoni) on waving branches, and there it definitely appeared to be cryptic behavior. It would be interesting to see if the

waving motion of water snakes changes with the presence and intensity of the wind.

Leaving the water. Another locomotory adaptation of water snakes is the method of leaving the water without making a wet track which might betray the basking snake to a predator. I watched several snakes come out of the water and none left tracks. A snake typically thrusts the anterior 1/6th of its body out onto the rock or shore. The base of this forward part is resting on the water line, which, in running water, fluctuates. The head and neck are raised about 2 cm from the rock. The posterior part of the body swims slowly forward to keep the snake from sliding down the rock while the forward part of the body dries in one or two minutes. Then another part of the body is drawn up and gets dry, and so on. Each part of the body is first drawn up parallel to the water line, so any wet mark it leaves is continuous with the water line. When the snake leaves the water completely, only a small bump in the water line remains, and this dries quickly. I timed the process of leaving the water for five different snakes. The times ranged from 3.7 minutes to 6.9 minutes with a mean of 5.1 minutes from the time the snake contacted the rock until its tail was out of the water. After the drying process, the snake usually

coiled or curved on the rock.

Escape behavior

Escape to water. Water snakes usually escape to water when alarmed or attacked. Several were observed in the clear water at Tremont, where they could be seen after they slid in. Locomotion across the stream was slow but constant. It was similar to that described above for terrestrial locomotion; the snake curved its body around rocks and appeared to flow along. Since all parts of the body followed the same path, and the reflections of the water distracted the eye, it was almost impossible to keep sight of the snake. After it disappeared behind one rock it could expose itself farther on and not be seen. A predator or collector might search for the snake under the rock that it first went to, and the snake has time to go to a safer place. In all cases observed, the snake surfaced on the opposite bank, usually up or downstream rather than straight across.

Captive snakes had a pool about two feet in diameter. When they escaped to the water they usually surfaced across the pool. Occasionally they would surface for an instant and then duck again to reappear at a point 90° around the pool. In these cases I could perceive no stimulus to make

them duck again, and they may have come up for an exploratory purpose.

Cues for escape behavior. The cue for escape behavior is visual or vibrational. Some authors have hypothesized that vibrational stimuli are received by the ventral surface of the snake. Wever and Vernon (1961) have examined snakes' hearing ability and determined that snakes can hear some low frequency sounds (from 100-700 cps) which are received by the quadrate bone. My observations did not confirm or contradict the existence of either vibrational or auditory reception. I feel that, at least for the Tremont snakes, the smell of humans does not elicit escape. This leaves visual stimuli as probably the most important. Both captive and wild snakes approached me if I was still, but a small motion, such as shifting binoculars, alarms them. On two occasions snakes have crawled over my leg as I sat observing them. On one of these, a snake came out of the grass, crawled over my instep, and went on. Five minutes later, another came from the same place, followed the same path over my foot, and disappeared. Some time later, I observed a group of snakes near me that may have been a mating aggregation. It is possible that the first snake left a scent trail on my foot, and that the second was following

it. On many less startling occasions, wild snakes came within one meter of me as I sat quietly.

The snake's response to visual stimuli differs according to the situation. One snake was observed for several hours basking on a rock one foot away from the water. Six times it was approached by a very active Louisiana Water Thrush and did not move. Twice it was approached by a muskrat eating water weeds, and both times the snake slid into the water. The different response may have been due to the different sizes of the approaching animals. Noble (1937) noted that snakes generally approach a "small" moving object and escape from a "large" moving object. The example of small object was another snake; presumably "small" meant not larger than a conspecific. I noticed an approach response in the wild-caught snakes. If I sat quietly in front of their tank and slowly moved a pencil, the snakes would approach me and push against the glass. When I moved my body or head, they ducked into the water. Speed of motion seemed to affect response also. The same pencil moved rapidly or jerkily elicited escape, but the return of the snakes seemed quicker. This could be tested quantitatively.

The snake's position and camouflage may also affect its escape behavior. A snake was in tall grass with just its head exposed, and in three instances, a person walked

along a path about three feet from the snake. The snake did not move. Later the snake moved down onto an exposed rock to bask, and when the same person walked along the path, the snake slid into the water.

Mimicry. If crypticity and active avoidance of attackers fail, a water snake has other escape behaviors. When cornered, snakes flatten the head and body, thrust out the quadrate bones, and assume a striking position. It is possible that this is a form of behavioral and morphological mimicry of poisonous snakes, as well as a general enlarging of the snake. Many human attackers associate large triangular heads of snakes with venom, and other animals may also respond to this signal. Three poisonous members of the Family Crotalidae share much of Natrix sipedon's range, and these have enlarged 'cheeks' to house their poison glands. Ditmars alludes to this mimicry in his 1946 statement: "It /sipedon/ is often called mocassin and is thought to be poisonous. In fact, its sinister aspect when cornered is anything but reassuring, for it will flatten its head and body to a remarkable degree, when it strikes viciously at moving objects within its reach."

Striking. If a water snake attacks, the jaws often miss entirely or touch the attacker without a strong bite.

However, if the attacker actually grasps the snake, it will bite hard and draw blood. Sometimes the snake leaves teeth in the wound. I have observed that hand held snakes will attack objects moving above them (hands or pencils), but will not attack objects moving below their heads.

Cloacal Secretion. Another response to being grasped is the production of cloacal secretion. This fluid is often mixed with fecal material, and to humans, it has an objectionable odor. It is sprayed out or rubbed against an attacker. A releaser for this behavior may be contact of the vent to a surface. The snake often winds around the arm of a collector and rubs the secretion onto the skin, moving the vent back and forth. I found I could easily collect the secretion from snakes by stretching them over a glass plate and rubbing the vent against the plate. However, some adult snakes sprayed the secretion with no contact of the vent against a surface. The young (one year old) snakes did not spray the secretion. As well as repelling an attacker, it may also help the snake become more slippery and wiggle out of the grasp of the attacker. Some authors have suggested that the cloacal secretion is an alarm pheromone in snakes (Brisbin, 1968). Pilot observations of Natrix sipedon have shown that the snake perceives the odor, but has given no

evidence for or against the pheromone theory.

Tail autotomy. Another escape mechanism is tail autotomy. Several snakes were caught at Tremont which had lost parts of their tails, from two to ten cm posterior to the vent. Most water snakes, when grasped by the tail, twist their bodies rather than randomly thrashing them. On two occasions I was holding a twisting snake and the tail came off in my hand. One tail was kept and it writhed slowly for 35 minutes. An objectionable odor, something like rotten tunafish, came from the tail, though no cloacal secretion had been produced. The odor was similar but not identical to that of the cloacal secretion. The two snakes were both mature females. Autotomy would be especially advantageous to an ovoviviparous snake, which carries the young in the body for three to four months.

V. FEEDING BEHAVIOR

Hunting

Snakes at Tremont often "hunted" after a period of basking in the morning. The behavior was interpreted as hunting because: (a) laboratory snakes which normally ate several pieces of fish would perform this behavior after being given one piece of fish; (b) one snake was captured

after this behavior was observed and disgorged food which had not been digested at all. Following is a description of a typical hunt at 8:30 a.m., July 3, 1971. The temperature was 20°C. A small snake, probably two years old, appeared swimming downstream one meter from shore. It swam in and paused for five minutes with its head on a rock, and then ducked under the water. It moved around the rocks, touching them with its nose on the tops, sides, and bottoms. More time was spent under the overhangs than on other parts of the rocks. Some tongue-flicks were seen, but they were difficult to see with the reflection of the water and they may have been constant. The snake moved out to a depth of about one meter and then started back. It surfaced at intervals of from six to thirteen minutes. It released air bubbles about every three minutes. The snake passed within 30 cm of a large feeding sculpin (Cottus sp.) of about 15 cm, but did not appear to respond to it. The snake was caught by hand and no large food could be felt in the stomach. Its snout-vent length was 19.5 cm, which is within the range of the length of a two year old snake (Brown, 1940).

This hunting behavior was observed many times, involving snakes of all sizes. One snake was captured after observations and disgorged three almost metamorphosed toads.

Fishing

Water snakes in the lab and in the enclosures were observed to "fish" in the water by opening their mouths to a fifty degree angle and then thrashing around until they contacted something. Sometimes there was some forward motion, but usually the snake remained in one place. When an object was contacted, the thrashing stopped; if the object was another snake or a part of the same snake, the mouth was closed and then opened again. No swallowing was started. If the object was a fish, the snake bit it, struggled with it, then started swallowing motions. The thrashing behavior began when a fish was introduced into the tank and continued until the fish was caught. One large snake often restarted the thrashing after the fish had been eaten; presumably a chemical cue was still present in the water. This type of feeding behavior was only observed with fish. It was described for wild N.s. sipedon and other Natrix species feeding at night by Evans (1942). Evans noted that this behavior was only seen in small water bodies and shallow riffles. I may have observed this only in captive snakes because: (1) I did not do night observations and Evans' observations were mostly at night with flashlights, (2) the wild snakes I observed were all active in large water bodies with much cover (boulders) for pursued fish.

Aimed attacks

For prey other than fish, attacks were aimed, even if the prey were swimming in the tanks. Aimed attacks were observed in attacking and eating toads (Bufo sp.), tadpoles (Rana catesbiana) and salamanders (Desmognathus fuscus). The attack was aimed at the prey only when it was moving. In a typical case, a live toad was dropped onto the gravel in a cage containing three mature Tremont N. sipedon. They were all resting under a piece of bark 75 cm from the toad. The toad hopped once and all three snakes came out quickly so that the heads were about 30 cm from the toad. It remained still and the snakes started searching (moving the head and first one-third of the body and tongue-flicking) as if unaware of the actual location of the toad. The toad hopped again and the snake with its head nearest the toad oriented at the instant of the hop and struck. It appeared that the mouth opened after the strike had been initiated, which makes this behavior quite different from the "fishing" behavior. The other snakes oriented as the first struck, but the first removed itself and the toad from their view.

Food running

Often in a cage with two or more snakes, a snake would catch a prey animal and "run" with it to prevent another

snake from grabbing it. In most cases, this was only observed when another snake was actually pursuing a runner. However, one snake consistently ran with its food, no matter where its cage mate was; this was a possible indication of subdominance. Typically, a snake running with food held the food out of the "reach" of a pursuer by holding up about one-fourth of the body.

Tongue-flicking. Three 8mm films were made of snakes in a glass tank with a super 8 Minolta. The snakes were presented with different stimuli, and tongue-flicking was filmed at 32 frames per second. 112 complete tongue-flicks were recorded. Incomplete tongue-flicks (those in which the start or finish was not filmed, or which were obscured by a dark background) were not recorded. Figures 1, 2, 3 and 4 were drawn from stop-action projections of tongue-flicks. The snakes used were a Tremont male (snout-vent length, 49 cm), a Tremont female (snout-vent length 64 cm) and a laboratory one year old snake (snout-vent length, 19 cm).

The tongue-flicks in Natrix sipedon vary in several ways:

1. Length of tongue extension from mouth. The shortest can not really be called an extension; the mouth opens slightly and the tongue tips come out of the tongue sheath to a point where they can just be seen. Presumably they can pick up a limited amount of chemicals from the air. This is a very "safe" tongue-flick; it will not draw attention to the snake. The tongue-flick which appeared to be the longest extension on the films was drawn (Figure 3f). The tongue extension is 8 1/4 times the distance from the lower rim of the orbit to the jaw.

2. Position of the tips of the tongue. The tips can

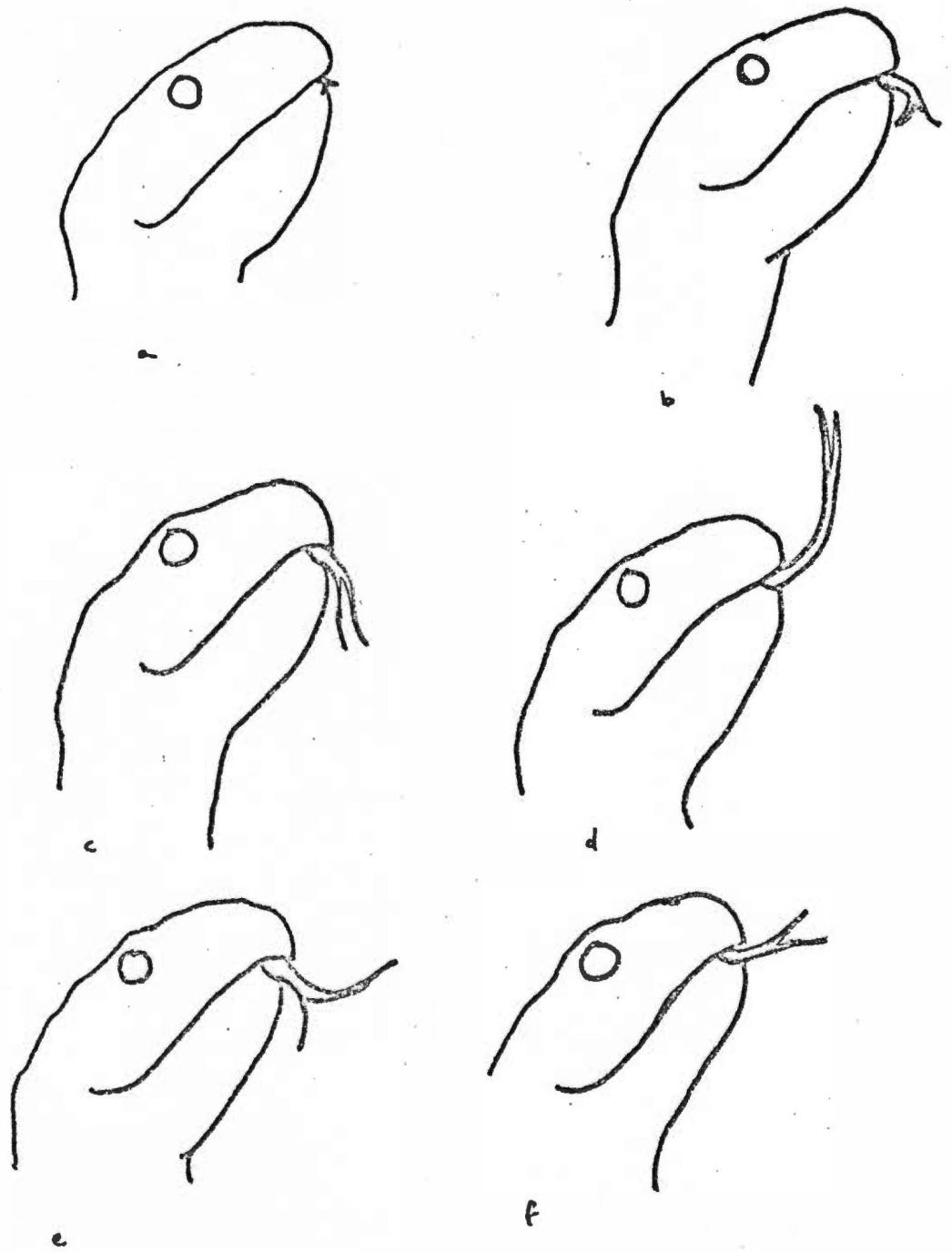

Figure 1. The first six frames of a tongue-flick of a male Tremont Natrix sipedon. The snake is in an "alert" position, as described in the text. These were drawn from a 32 frame per second film.

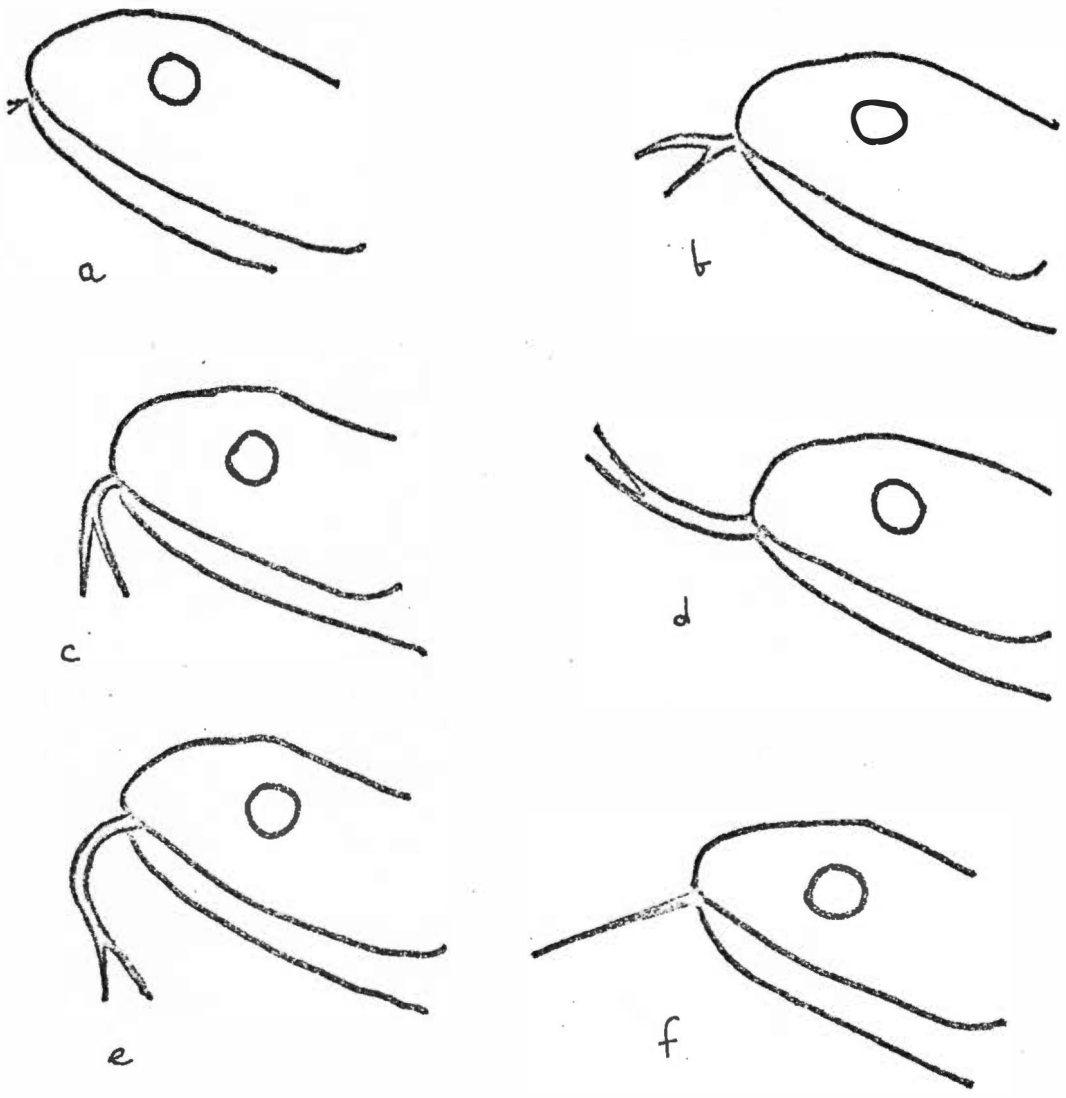

Figure 2. Six frames from a tongue-flick of a female Tremont Natrix sipedon. The snake had just eaten a fish, and after this sequence, it ate another fish. These were drawn from a 32 frame per second film.

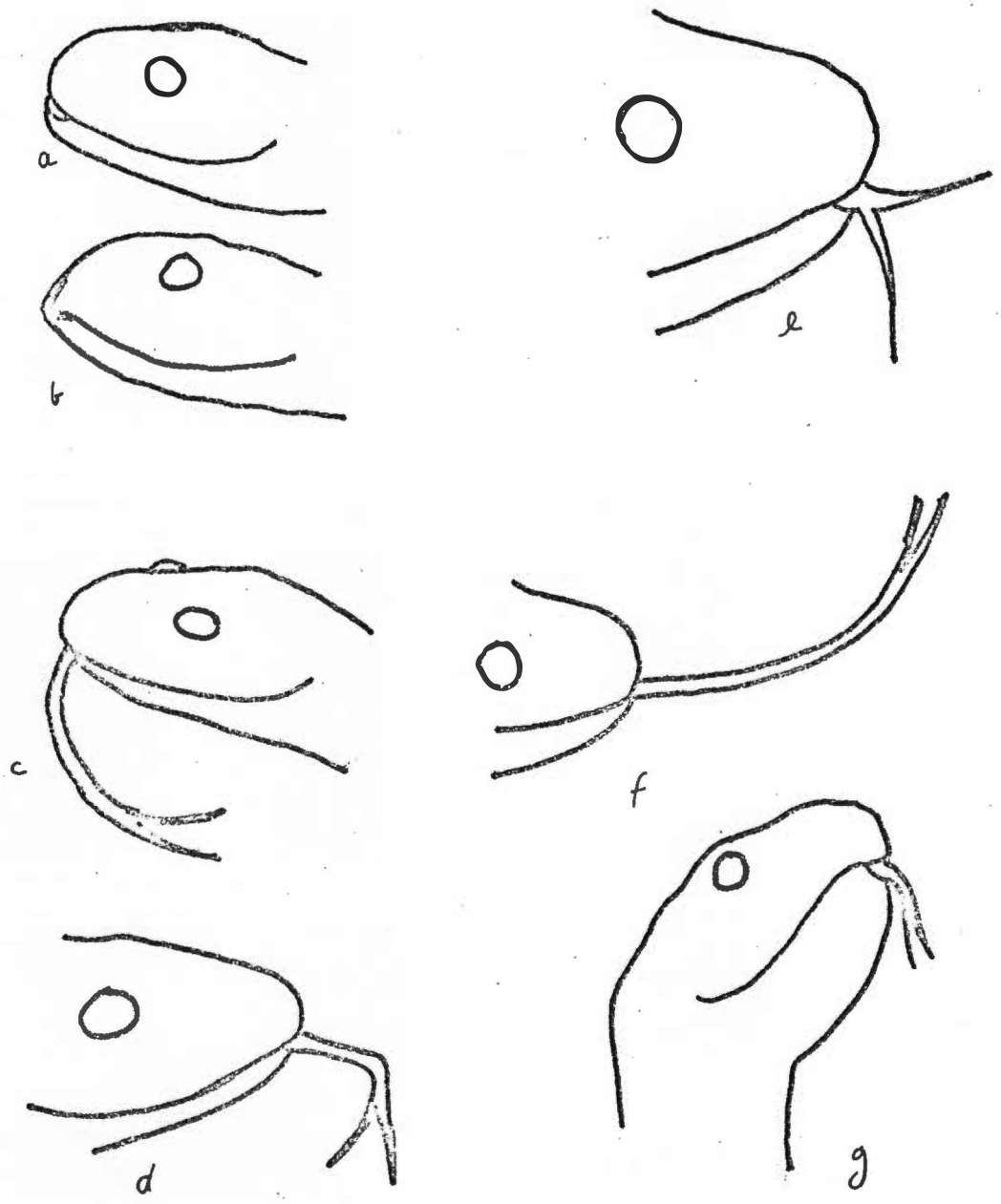

Figure 3. Some positions of the tongue in tongue-flicking in Natrix sipedon (a). The mouth just before a tongue-flick, showing the opening through which the tongue extends. (b). The same snake as in (a) just after the completion of a tongue-flick; the mouth is closed. (c). The tongue is curving to the left. (d). An angle of the tongue. (e). The tips of the tongue are spread to about 90°. (f). The longest extension of the tongue. (g). Downward curvature of the tongue.

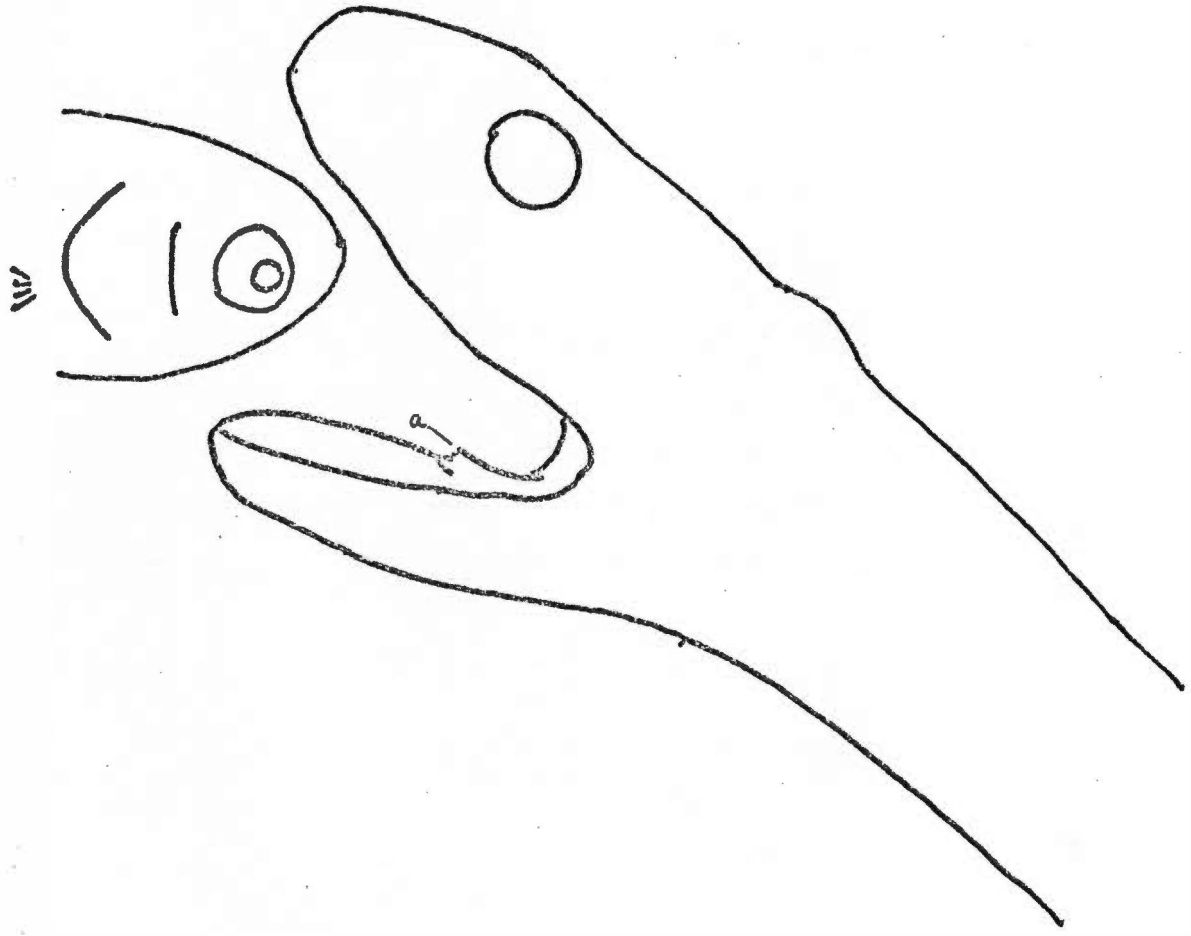

Figure 4. A Tremont female Natrix sipedon about to eat a goldfish; (a) indicates tongue sheath with the tongue completely withdrawn.

be touching each other or spread to an angle of 95° (Figure 3e).

3. Extent of up and down motion. These are shown in Figures 1d and 3g.

4. Degree and direction of turning of the tongue. The most curvature is shown in Figure 3c; it was hard to see this because the film was taken from a side view. Top view films would be useful.

5. Length of tongue-flick. Of the 112 tongue-flicks observed, the mean length was 0.49 sec. Some of the tongue-flicks measured could be categorized as to situation. Eight tongue-flicks occurred just after eating a fish and before eating another one. Sixteen tongue-flicks occurred between attacks on my finger. Thirteen tongue-flicks occurred while the snake was being held by hand. The mean times of these tongue-flicks are in Table II. This indicates that in times of stress or danger, tongue-flicks are longer than in the feeding response.

A quick out and in motion of the tips preceded three of the tongue-flicks. In each of these cases, the snake was in an "alert" position, with the anterior 1/4 of the body raised up straight. The tongue-flick lasted one frame, that is, no more than 1/32 of a second. It may be used at times that it might be dangerous for the snake to tongue-

TABLE II. Mean and ranges of duration of tongue-flicks of *Natrix sipedon* which occurred in different situations

SITUATION	N	MEAN DURATION	RANGE
The snake has just eaten a fish and is about to eat another	8	0.25 sec.	0.11-0.37 sec.
The snake is attacking a finger	16	0.44 sec.	0.25-0.62 sec.
The snake is grasped and hand held	13	1.17 sec.	0.75-1.61 sec.
All tongue-flicks recorded	112	0.49 sec.	0.03-1.61 sec.

flick and thus become conspicuous.

Figure 2 shows a tongue-flick sequence of a snake about to eat a fish. Each diagram represents one frame. Figure 1 shows a sequence while the snake is in the "alert" posture. Some frames were left out which were basically similar to those shown. Figure 3 shows some other variations of tongue position.

VI. DISCUSSION

My observations of wild Natrix sipedon add some details to the scattered writings about snake behavior. I limited my reporting to observations of feeding and defense behavior for two reasons:

1. Knowledge of these behaviors was the most helpful in carrying out the extract response experiments and interpreting the behavior. This was especially important in testing wild-caught snakes, which were not habituated to a captive situation and the presence of humans.

2. Feeding and defense behavior were relatively easy to observe, since they occurred frequently. Escape was the commonest, because the snakes were extremely sensitive to my presence, and that was the behavior I most often saw and could reliably elicit. The observation period (three months) was not long enough to get adequate information on less

frequent behaviors, such as courting, mating, giving birth and hibernating. I feel that at least two seasons would be necessary to get valuable data on these behaviors; the first year could be spent in finding out when and where the behaviors occurred, and the second year could be spent doing detailed observations. I observed some social behavior, but the volume of observations was too low to make reasonable interpretations. Since the snakes are so difficult to see, my limited observation period did not produce a lot of data. Also, the snakes move slowly; I spent many hours watching snakes doing nothing.

Consequently, my observations are in no way an attempt to develop an ethogram of Natrix sipedon; mating and other social behaviors were ignored; homing and phenology were not considered. Mating and aggregations of natricine snakes have been studied by Noble (1937) in the laboratory. Social behavior such as dominance and inter-individual distance have not been investigated but I feel that they are worthy of attention. Noble also studied the role of the chemical senses in mating, feeding and aggregating, and investigated mating pheromones. More work could be done on pheromones and their effectiveness in trailing and other behaviors.

Fraker (1970) demonstrated that N.s. sipedon has a definite home range and homes consistently when removed

short distances (0.2 miles - 0.5 miles). The larger snakes had a greater return rate than small snakes. He speculates that olfaction plays a role in the homing response, since the snakes move randomly until they reach their home range rather than heading straight for it. If that is true, then it is possible that the prey animals peculiar to a home range provide an olfactory stimulus which keeps the snake in it. This would not have much bearing on snakes in an area like Sterchi's, where one pond or one area of the creek is not much different from another. However, in areas like Tremont, where a snake may get swept downstream by heavy rain, species composition changes quickly with change in altitude, substrate, and local plant communities. It is to the snake's advantage to be able to return, since it may be familiar with the cover in the area.

I have seen no work on phenology as such, though notes are included in many papers about the affects of season and temperature on reptiles in general and on Natrix in particular (Bellairs, 1970; Brown, 1940).

These observations, even though limited, gave me some understanding of the behavior patterns of feeding and defense of Natrix sipedon. Unfortunately, I did not make any observations which directly support my experimental results. However, the observations helped me in interpreting behavior

in the laboratory and in providing an experimental situation that would alarm wild-caught snakes as little as possible. The observations also gave me a greater understanding of the ecology of the snakes. The experimental work involves one aspect of the ecology, the prey extract response, which indicates food preferences.

CHAPTER III

FOOD PREFERENCE EXPERIMENTS

I. GENERAL PROCEDURE AND AIMS

The food preference of three groups of experienced Natrix sipedon was tested and compared. The groups are as follows:

1. Lab reared one year old snakes. These had been fed the same food, feral goldfish (Carassius auratus), until tested. They were born on August 9, 1970, of a female obtained from Midwest Reptile Company, Fort Wayne, Indiana.

2. Wild-caught snakes from Tremont. Since these snakes were caught at different times from June 1st, 1971, until the beginning of testing in August, 1971, it was necessary to feed them every four days, and they also received goldfish. If they had not been fed, a large variation in motivational state might have affected the data.

3. Wild-caught snakes from Sterchi's bait hatchery. These were also caught over a period of time and fed goldfish.

All of these snakes were tested for their response to extracts of the skin of prey species presented on cotton swabs, and the tongue-flicks and the latency of attack were quantified. The purpose of the testing was to determine what

affect the snake's experience had on its response to the chemical stimuli.

II. EXTRACT PREPARATION

Skin extracts were used to test the relative preference of Natrix sipedon for various food items. The extracts came from live prey specimens, which were washed, dried, weighed, then stirred in distilled water for one minute at 60°C. Twenty cc of water were used for each six grams of prey. The extract was then centrifuged for ten minutes at 3,000 rpm, and the supernatant fluid decanted into small vials with tight caps and frozen until thawed for use. The extracts made are described in Table II. 'Little River' refers to an area about two miles down from the observation area at Tremont in the GSMNP. All of the fish species collected at that site also occur at Tremont (Robert Stiles, pers. comm.). The extracts from the prey animals remained frozen until the beginning of each series of tests, then were kept in the refrigerator between tests of the series. No extract was used for more than three and one-half days as it was noted that after four days some of the extracts that I could smell developed a rotten odor. Burghardt and Hess (1968) noted that the earthworm extract lost its effectiveness after four days after preparation, even though

TABLE III. Extracts prepared for testing the extract response of *Natrix sipedon*

Prey Species	Symbol	Where Found	Date Prepared
FISH			
Campostoma anomalum	FCan	Little River	June 8, 1971
Carassius auratus	FCau	Bait supply	June 4, 1971
Cottus carolinae	FCc	Little River	June 8, 1971
Etheostoma ruflineatum	FEr	Little River	June 8, 1971
Notropis coccogenis	FNc	Little River	June 8, 1971
AMPHIBIANS			
Bufo fowleri	TBf	Sterchi's	July 2, 1971
Desmognathus fuscus	SDf	I.C. King Park (Knoxville)	June 4, 1971
D. monticola	SDm	Cades Cove GSMNP	June 4, 1971
Gastrophryne carolinensis	TGc	Sterchi's	July 15, 1971
Plethodon glutinosis	SPg	Cherokee Wood Lot (U-T)	June 4, 1971
Rana catesbiana (tadpole)	FrRc	Sterchi's	July 6, 1971
Rana pipiens	FrRp	Tremont	June 4, 1971
OTHER			
Mus musculus (baby)	Mm	UT Psych. lab	June 4, 1971
Orconectes sp.	Or	Little River	June 8, 1971

refrigerated.

III. HOUSING OF SUBJECTS

The wild-caught snakes were tested with a slightly different procedure than the laboratory-reared snakes for the following reasons:

1. The laboratory snakes were relatively tame because they had lived in glass tanks for one year. Though they had not been handled, they were accustomed to a person moving around, removing the covers to their tanks, cleaning, and feeding, and they were no longer startled at these stimuli. It was noted that the wild-caught snakes became alarmed at these stimuli. They assumed aggressive postures, made attack movements directed at a person passing the tank, or escaped under a towel. They did not often eat when people were present and moving.

2. All of the lab snakes had been fed with forceps, and although this occasionally elicited an aggressive response, it usually did not. The wild-caught snakes normally refused food from forceps. The main difference between the procedures used for the wild-caught and laboratory snakes was in the housing.

Housing for laboratory-reared snakes

The laboratory snakes were tested in six-quart 33 X 9 X 17 cm glass tanks with a flat glass plate over the top of each. Lighting came from a window and an overhead fluorescent bulb. Each tank had a five by ten cm section of paper towel and a small plastic dish of water which was kept 2/3 full at all times. Index cards were placed between and in front of the tanks to keep the snakes visually isolated from each other and from most of the motions of the observer. One hour before testing, the glass plates and paper towels were removed; the tank was wiped if it was wet or dirty; and the petrie dishes were changed to remove any residual odor from a feeding. The snakes were all the same size and seldom adventured up the sides of the tank, so the glass tops could be left off throughout a testing session.

Housing for wild-caught snakes

The wild-caught snakes were housed in four 30 cm cubical wooden boxes with a plexiglass front window and a screened, hinged top secured with a hook. White index cards were taped to the floor of the box for a lining to increase visibility. A plastic petrie dish of water was placed in a corner of the box and was often moved about by the snakes. The boxes stood on the floor with the windows facing a

fluorescent study lamp which was the only light in the shaded room. The lamp was under a table and a cardboard curtain prevented light from reflecting around the room. The observer sat on a chair behind the boxes. The experimental set-up is shown in Figure 5. During testing, the screened tops were lifted up toward the observer, further shielding the observer from the snake's view. Four snakes were tested in each series, and since the larger snakes could easily leave the testing box, the screen tops were quietly closed after each trial.

IV. TESTING PROCEDURE

Managing and ordering of extracts

When the snakes had been prepared for testing the extracts were taken out and left at room temperature for 40 minutes. A 15 cm cotton swab and a paper towel were assigned to each extract vial, and these were set out on a table in the order of testing. Every snake was tested once with every extract in each session ("Session" here refers to continuous trials over a period of two to four hours occuring either once or twice a day). For the first snake in each series of sessions the extracts were ordered randomly with the constraint that if two fish stimuli came together, another type of stimulus, such as anuran, was inserted between them. The

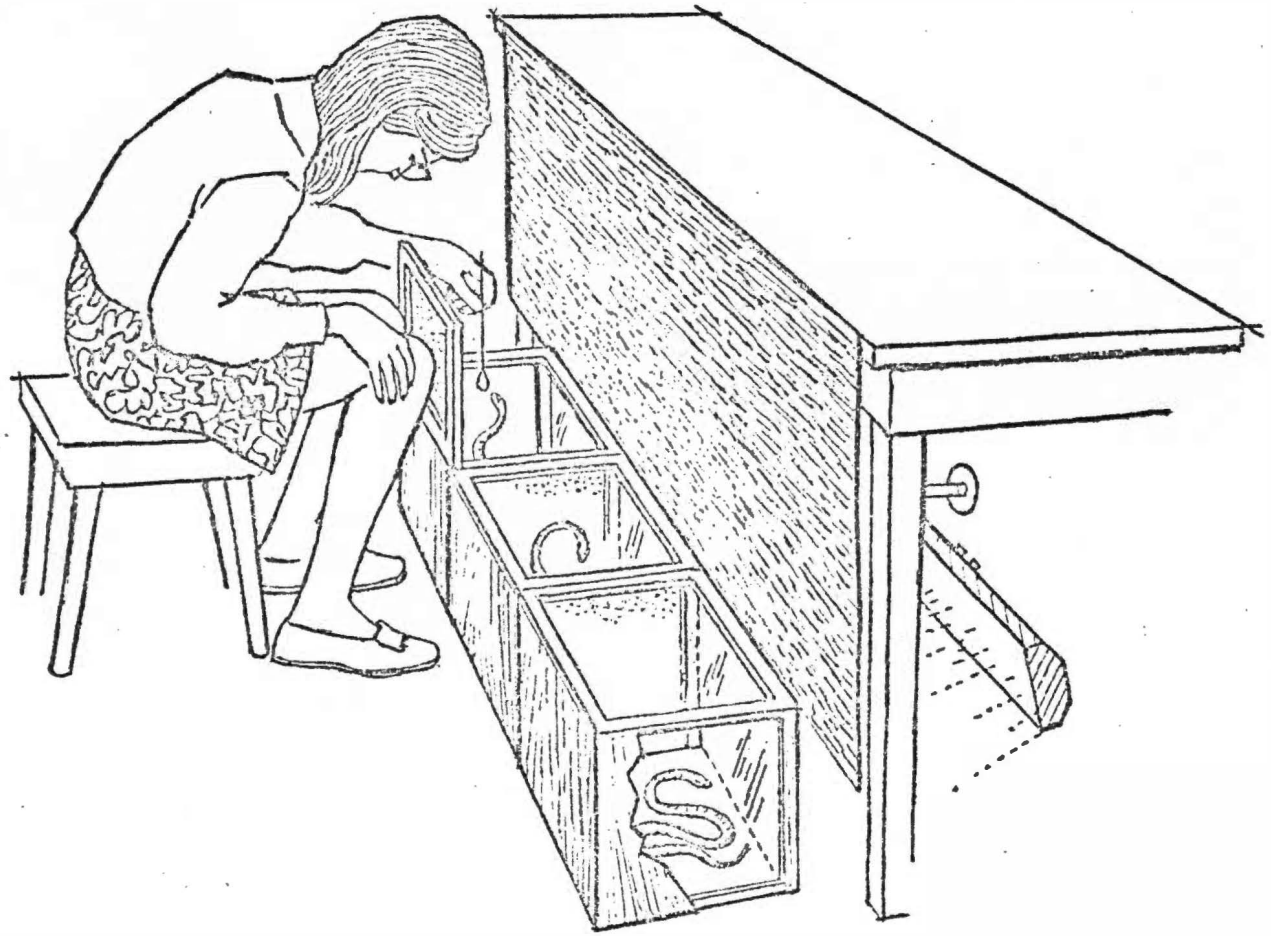

FIGURE 5. Laboratory set-up for testing wild-caught snakes. The light under the table is the only light in the room, so the experimenter is in shadow. In actual testing, four boxes were used.

order for the second snake was the same except the first extract was dropped into last position, and so on. At each successive session, the first stimulus for each snake was dropped to last position.

Trials

A trial lasted two minutes. It began when the swab, having been dipped into the extract vial and shaken once, so that extract could not drip into the tank, was positioned about 2 cm from the snake's snout, slightly to one side. The trial ended if the snake attacked (defined by opening its mouth to an angle and lunging toward the swab) or when two minutes elapsed. Opening the mouth to any angle without lunging was recorded as a gape. No attacks were seen with an angle of less than 30°. If an aggressive attack occurred, which is easily distinguishable by the flattening of the head and body, the trial was postponed for 20 minutes and then repeated. This only occurred twice, and so did not disrupt the testing schedule. For an individual snake at least twenty minutes elapsed between trials.

Scoring and recording

Total tongue-flicks were counted with a mechanical hand counter. Tongue-flicks that were not directed toward the swab were counted mentally and subtracted from the total

to get the recorded figure of "tongue-flicks to swab." Since the swab was not directly in front of the snake, it was not difficult to determine if the tongue-flick was directed toward the swab. Tongue-flicks toward the swab were recorded if: (1) the tongue turned toward the swab; (2) the head turned toward the swab and the tongue-flicks became straight; or (3) the snake approached the swab, overshot it, and tongue-flicked beyond the swab before returning to it. All others, including those in the third category if the snake did not return to the swab, were recorded as general tongue-flicks. If the snake attacked the swab, the latency of attack was recorded to the nearest second. A snake which did not attack was given a trial score of the tongue-flicks to the swab. If an attack occurred in a trial, the snake was given a score which consisted of its tongue-flicks to the swab plus 120 minus the latency of attack in seconds.

Statistical treatment

Results are presented visually on graphs which also indicate significant differences. The mean score and standard deviation were computed for the combined scores of all the snakes in each of the three groups for each extract. A critical distance was computed using the following formula:

$$SD\sqrt{2/N} = \text{Critical distance}$$

The graphs of the mean critical distance indicate significant differences where the critical areas of the two means do not overlap ($p < 0.05$). This method was used by Burghardt, 1969, when developing extract response profiles for newborn snakes.

In addition, a Wilcoxin matched pairs signed rank test was run for each group of snakes to compare the response to two fish extracts ($p < 0.05$) (Sokal and Rohlf, 1969).

The data graphed in Figures 6 and 7, which is the data for the wild-caught snakes, was given a square root transformation. The variances for the wild-caught snakes were much greater than for the laboratory reared snakes, probably because they were less accustomed to captivity. The square root transformation reduced the variance. The data was not transformed, however, in carrying out the signed rank tests.

Test series

Table III shows the tests that were carried out. The results of Food Preference (FP) A and B affected the choice of extracts for the rest of the testing. Some extracts that were not available for the early series were used later.

In FP A and FP B, every snake was tested once with each possible serial order of extracts. The scores for the extracts when at the beginning of the series and when at the end of the series were compared with a Wilcoxin matched pair-signed rank test and found to not be significantly different, so it was assumed that there was no appreciable order affect. In the following series of tests, the order was varied in the same way, but each snake got most of the possible serial orders, not all. Only eight sessions of testing were administered instead of the eleven that would have been necessary to give each snake each serial order.

TABLE IV . Food preference (FP) series carried out. Snakes were tested with the extracts listed. Symbols for extracts are as in Table II.

Series	Snakes	Extracts*	No. of Sessions	Dates	Temp. & Humidity
FP A	10 Lab	SDf, Mm, SDm, FCau, SPg, FrRp,	7	8-18 June	24°C-25°C 55-65%
FP B	10 Lab	FNc, F, Or, FEr, FCau, FCan.	7	29 June-12 July	25°C 58-63%
FP C	2 Tremont 2 Sterchi	SDm, FCc, TBf, FCau, FrRc, FNc, FrRp, FCan, FEr, TGc.	8	24-25 July	25°C-26°C 58-64%
FP D	2 Tremont 2 Sterchi	SDm, FCc, TBf, FCau, FrRc, FNc, FrRp, FCan, FEr, TGc.	8	29 July-1 Aug.	25°C-26°C 58-59%
FP E	2 Tremont 2 Sterchi	SDm, FCc, TBf, FCau, FrRc, FNc, FrRp, FCan, FEr, TGc.	8	12-15 Aug.	24°C-25°C 58-65%
FP F	8 Tremont (newborn)	SDm, FCc, TBf, FCau, FrRc, FNc, FrRp, FCan, FEr, TGc.	6	1-10 Oct.	23°C-25°C 50-61%

*A distilled water control was used in all sessions of all series.

V. RESULTS

Figure 6 shows the response profile for the laboratory-reared snakes. The lines for Carassius auratus are higher and do not overlap with other lines, indicating that the response to goldfish was significantly greater. A Wilcoxin signed rank test between C. auratus and the next highest fish extract, N. coccogenis was also significant. Intermediate lines on the graph were for extracts of other prey that N. sipedon reportedly eats (Table I, page 3). Table V shows that C. auratus received a much higher percentage of attacks than any other extract. The unexpectedly high response to M. musculus was due to one snake which consistently responded to mouse second to C. auratus. All other snakes' responses to mouse ranked sixth or seventh. Figure 7 shows the individual scores for each snake; as can be seen, Snake 8 is the only snake with an appreciable response to mouse extract.

Figure 8, with the data from the Tremont N. sipedon, shows a significantly higher response to Etheostoma rufilineatum, Cottus carolinae, Notropis coccogenis, and Campostoma anomalum than to Carassius auratus. The water control elicited significantly less response than any other stimulus. A Wilcoxin signed rank test between responses to C. carolinae and C. auratus also showed a significant difference ($p < 0.05$).

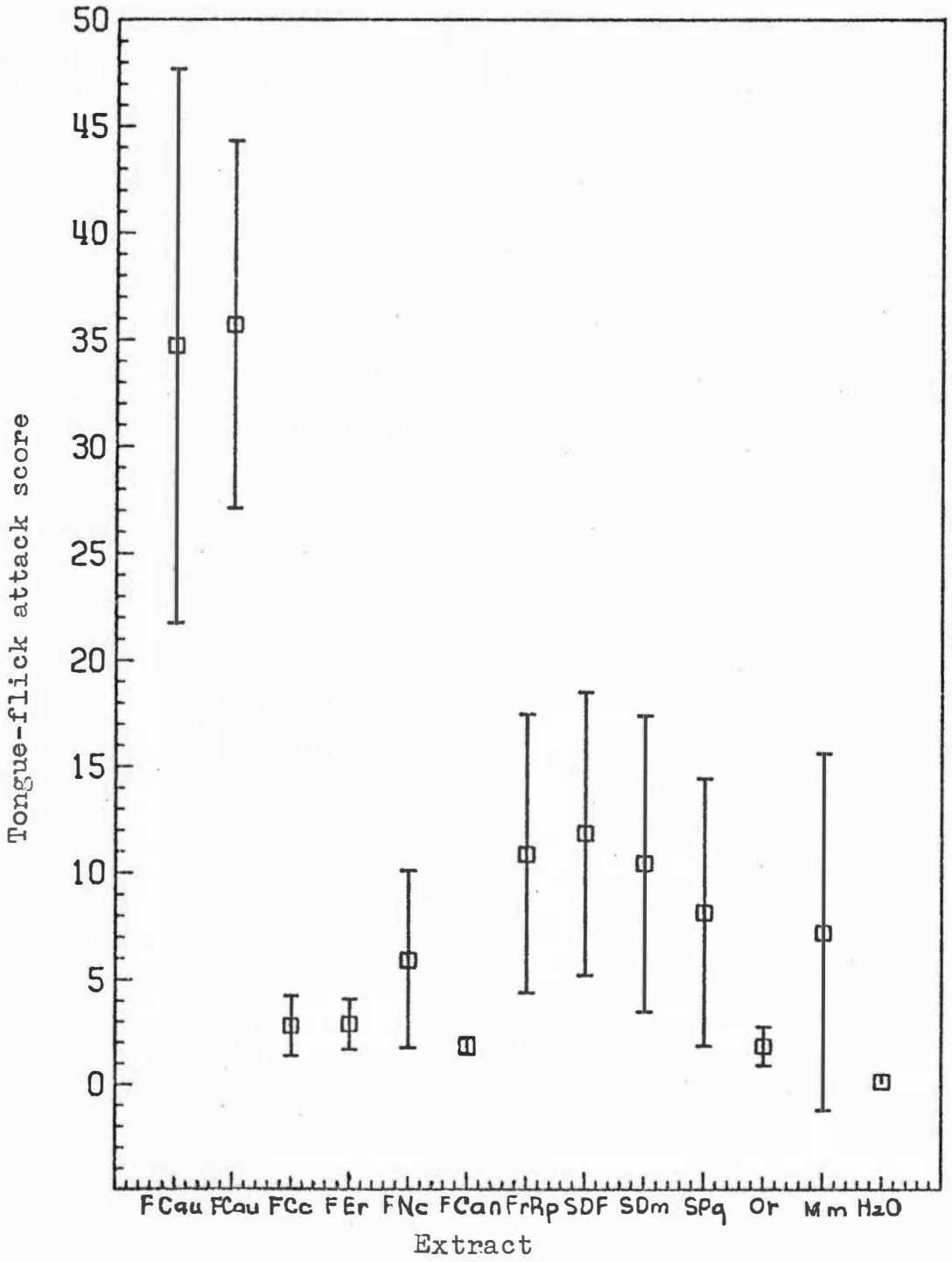

Figure 6. Responses of laboratory-reared Natrix sipedon to extracts of prey species.

TABLE V. Number and percentage of attacks of laboratory-reared Natrix sipedon when presented with prey extracts

EXTRACT			ATTACKS FOR LAB SNAKES	
			Number	%
FISH	FCau		21	30
	FCc		0	
	FEr		0	
	FNc		1	1
	FCan		0	
		TOTAL	22	
AMPHIBIANS				
	FrRP		3	4
	SDf		3	4
	SDm		2	3
	SPg		4	6
		TOTAL	12	
OTHER	Or		0	
	Mm		4	6
	H_2O		0	
		TOTAL	4	

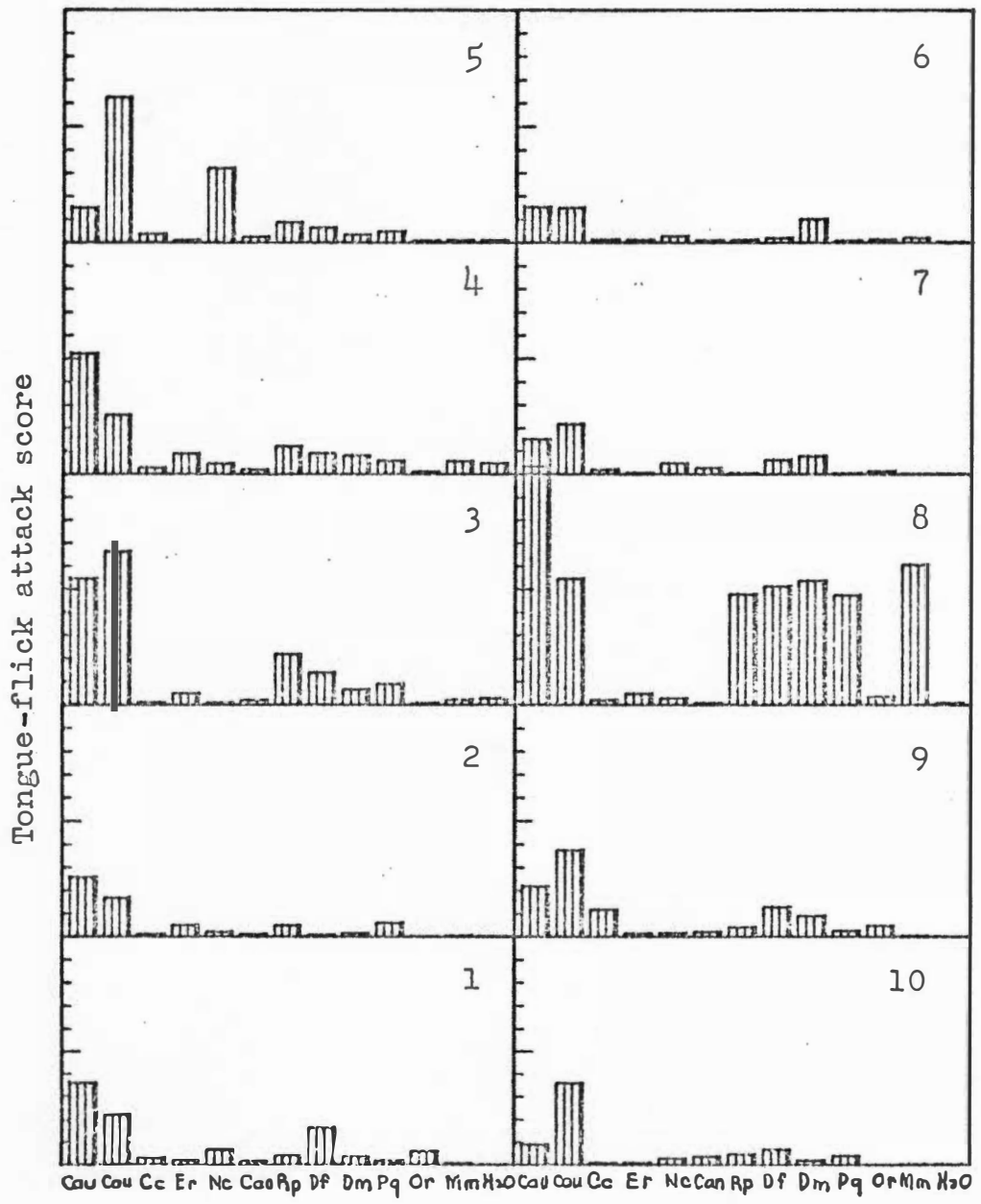

Figure 7. Responses of individual laboratory-reared Natrix sipedon to extracts of prey species. The scale for each box is 0-100.

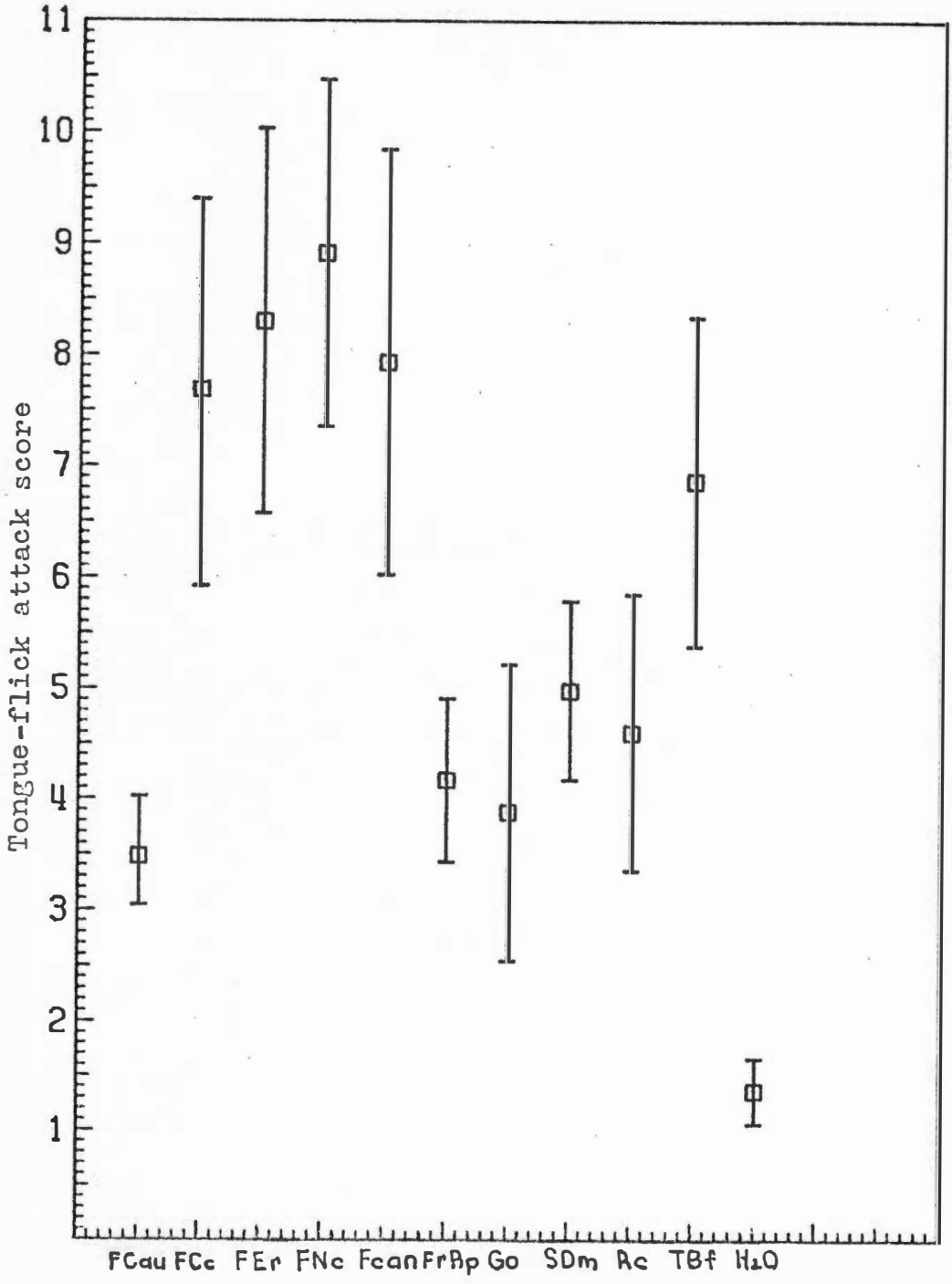

Figure 8. Responses of Tremont Natrix sipedon to extracts of prey species.

The attack scores (Table VI) show a higher percentage of attacks for the extracts which received a high score. One Tremont snake had a 100% attack score for N. cocogenis, E. ruflineatum, and C. carolinae. Another had a 100% attack score for E. ruflineatum. Figure 9 shows the individual scores of the Tremont snakes and shows their length and weight. Figure 10 shows a significantly higher response for Carassius auratus, than for any other fish species. Gastrophryne carolinensis, the narrow mouthed toad, did not elicit a significantly lower response. A Wilcoxin signed rank test showed a significant difference between the responses to C. auratus and N. coccogenis ($p \leq 0.05$). (For reasons explained later, formal comparisons were not made between responses to fish and amphibians.) Figure 9 shows the individual scores for each snake and compares these with those of the Tremont snakes. The Tremont snakes are clearly more responsive. Figure 10, which is on the same scale as Figure 8 for the Tremont snakes, also shows this. Table VI shows that the Sterchi snakes had a much lower general attack percentage than the Tremont snakes, and that C. auratus received more of the attacks. The general tongue-flicks, which are not shown in this data, were also higher for the Tremont snakes than for the Sterchi snakes.

TABLE VI. Number and percentage of attacks of the Sterchi and Tremont *Natrix sipedon* when presented with prey extracts

Extract	Attacks for Sterchi Snakes		Attacks for Tremont Snakes	
	Number	%	Number	%
FISH				
FCau	6	14	0	
FCc	0		14	33
FEr	0		22	52
FNc	1	2	24	57
FCc	0		21	50
TOTAL	7		TOTAL 81	
AMPHIBIANS				
SDm	0		1	2
TGc	0			
FrRc	0		2	5
TBf	1	2	10	24
TOTAL	1		TOTAL 12	
CONTROL				
H_2O	0		0	

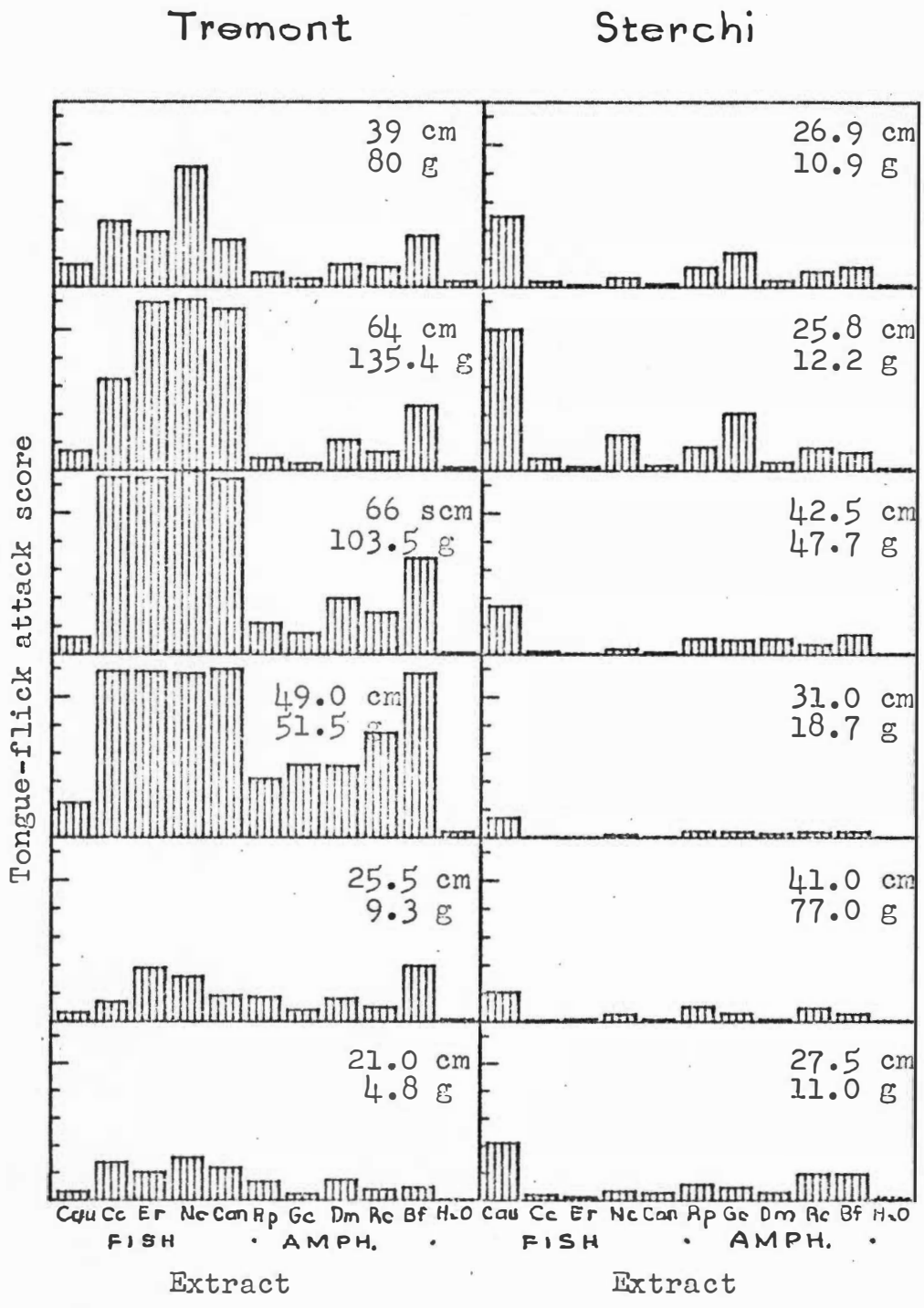

Figure 9. Responses of individual Tremont and Sterchi Natrix sipedon to extracts of prey species. The figures in each box are the snout-vent length of the snake mcm and the weight in grams. The scale for each box is 0-100.

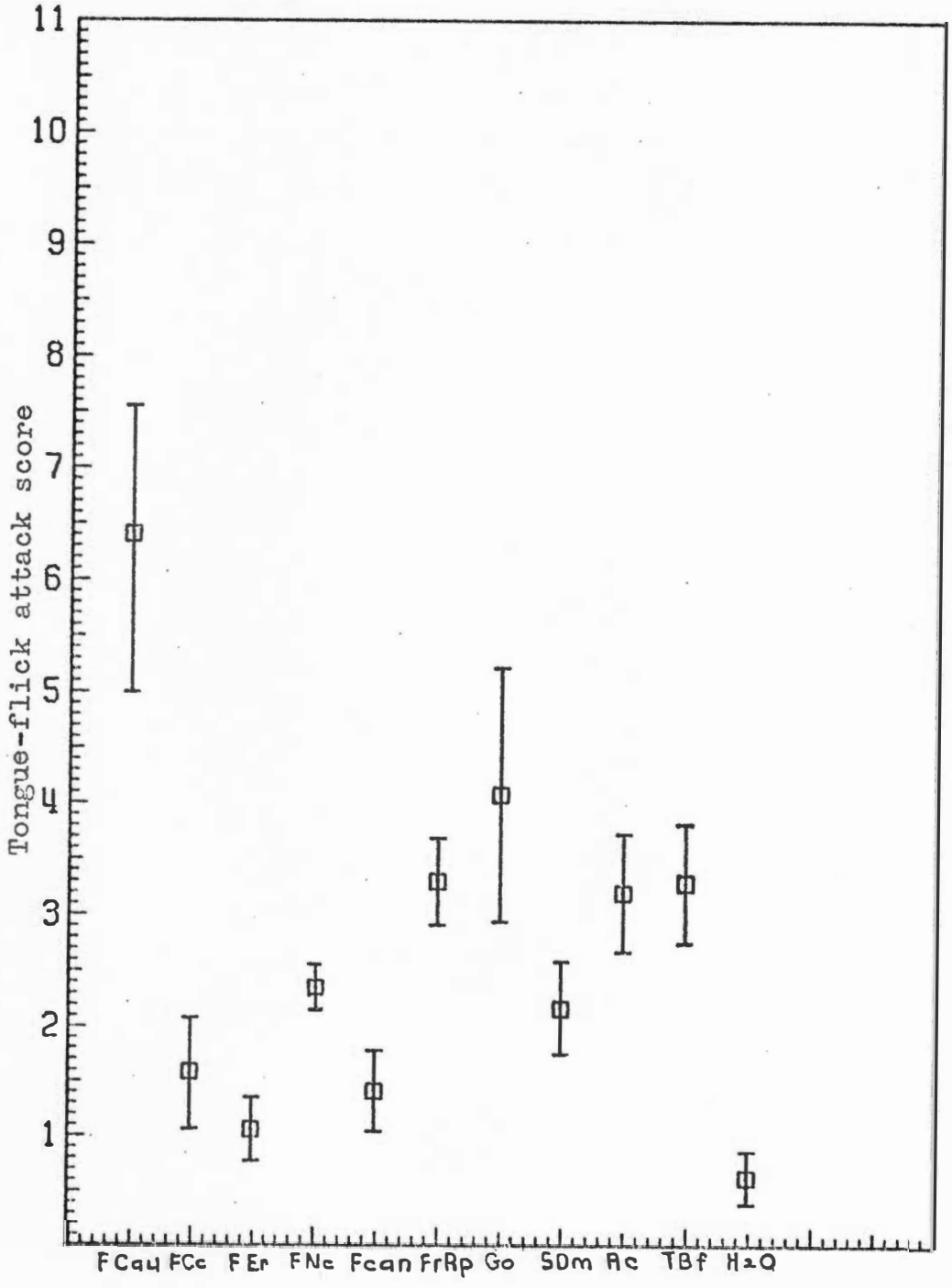

Figure 10. Responses of Sterchi Natrix sipedon to extracts of prey species.

Eight newborn snakes, born in the lab of Tremont parents, were tested with the same extract sequences as the Tremont and Sterchi snakes. The combined response profile and individual response scores are shown in Figures 11 and 12, respectively. The response profile is quite different from that of the experienced Tremont snakes. The highest response was to extract of Desmognathus monticola, but this was not significantly different from the responses to young Bufo fowleri, tadpoles (R. catesbiana), C. carolinae, N. coccogenis or C. anomalum. E. ruflineatum, C. auratus, and R. pipiens all received relatively low responses. No attacks occurred in this series.

The individual scores show that there is a large variability between the snakes of the same group. The variability is greater than among wild-caught snakes that were not litter mates.

VI. CONCLUSIONS

Food Preference A and B. In Food Preference I and II, Laboratory-reared Natrix sipedon responded more to the extract of prey that they had eaten for one year than to extracts of foods they had never eaten. The extract, of Carassius auratus, was presented with extracts from other fish species and amphibian species which have been reported

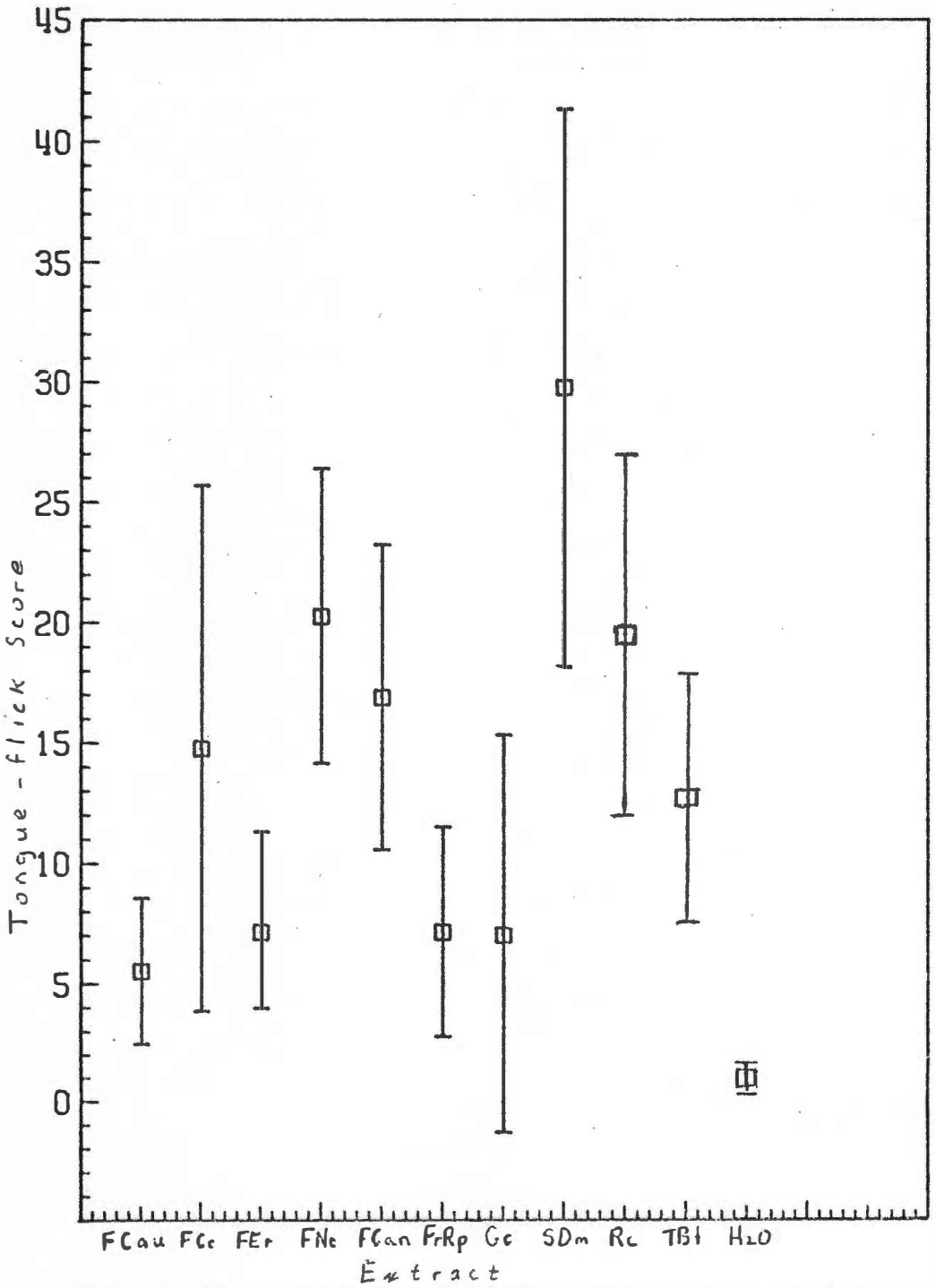

Figure 11. Responses of Tremont newborn Natrix sipedon to Extracts of Prey Species.

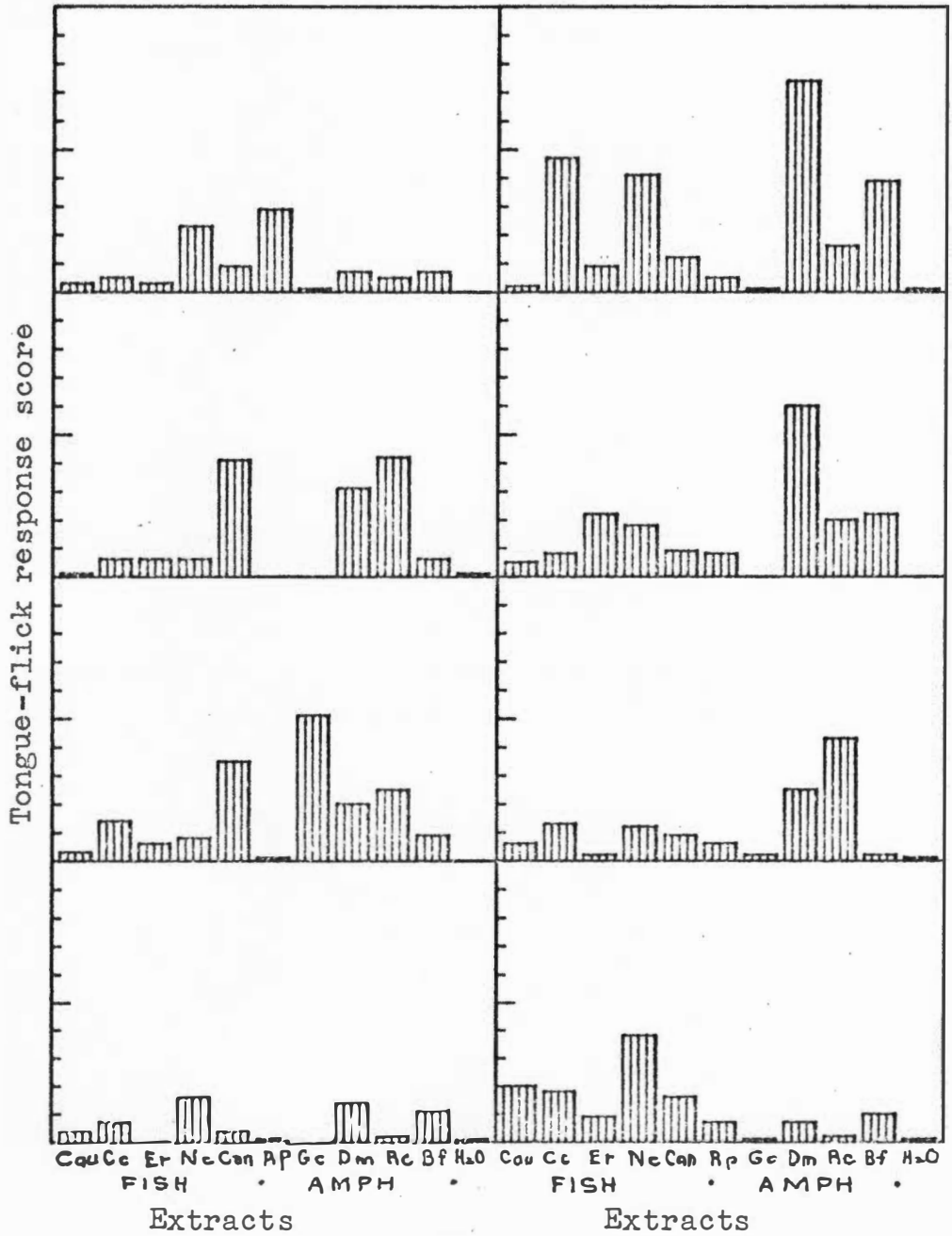

Figure 12. Tongue-flick scores of individual newborn Natrix sipedon from Tremont to extracts of prey species. The scale for each box is 0-50.

as prey for natural populations of N. sipedon. The snakes were not tested before their first feeding, so it is not known that their response had changed because of their eating experience. However, that may be assumed on the basis of the results of Fuchs and Burghardt (1971) that are described in the Introduction. They found that Thamnophis sirtalis (another natricine snake) changed in response pattern to extracts because of feeding experience. Also, Burghardt's (1968) response profile of newborn N.s. sipedon showed that frog extract elicited a greater response than minnow or goldfish.

The results here also indicate that the snakes are capable of distinguishing between different genera, and that they do not seem to generalize the "fish" stimulus. It would be interesting to do further work to see if snakes can discriminate between fish species in the same genus, but that was not done in the present experiment. The two members of the same genus used in the trials, Desmognathus fuscus and Desmognathus monticola, did not elicit significantly different responses.

An intermediate response was given by the laboratory snakes to extracts of species that they were reported to eat in natural situations, such as Notropis coccogenis, Rana pipiens, etc. These were reported from stomach contents by

Brown (1949) and others (See Table I, page 3). Lower responses were given to distilled water and to prey species that the snakes do not normally eat, Mus musculus and Crayfish. This may indicate that the intermediate level of response, to new prey extracts, is due to the innate preference of the snakes. It is advantageous to the snakes to retain the ability to respond to species-characteristic prey even while increasing the response to particular items, in case the ecology or prey availability of the snakes' home range changes.

The attack scores here are consistent with the combined tongue-flick-attack scores.

Food Preference C, D, and E. In Food Preference C, D and E, wild-caught snakes with unknown experience were tested with a group of extracts. Some of the extracted species were found in Tremont, some at Sterchi's, and at least two, Rana pipiens and Bufo fowleri are found in both areas. A Notropis species occurs in the creek at Sterchi's, but it is not N. coccogenis, and its population there is probably lower than that of C. auratus. The highest responses of the Tremont snakes were to extracts of prey found in the Tremont area; the highest response of the Sterchi snakes was to extracts found from prey at Sterchi's. The Tremont snakes

had a relatively low response to the C. auratus extract, even though they had eaten this species in the lab immediately prior to the testing sessions. This indicates that early experience is more important in determining the response than is recent experience. All of the Tremont snakes had at least one meal of C. auratus, and none had more than four (the meals were spaced four days apart, and consisted of one or two fish).

It would have been useful to include some more fish species from Sterchi's, such as the other bait fish he raises or some of the wild creek fish. But C. auratus was abundant in some of the ponds and also in the creek. I observed that many of the creek minnows were faster swimmers than the goldfish. Also, many goldfish died, and the bodies were available to the snakes. It is safe to assume that C. auratus is one of the prevalent food species for snakes at Sterchi's. Fraker (1970) also worked with snakes from a fish hatchery, and observed that N. sipedon ate equal amounts of frogs and goldfish.

The attack scores are consistent with the combined scores. Both sets of scores indicate that the Tremont snakes were much more responsive, assuming that at least one extract was a strong releaser for both populations. Also, the response to the control was greater for the Tremont snakes.

this difference could be for several reasons, and it may be correlated with my difficulties in observing snakes at Sterchi's as compared with snakes at Tremont. One possibility is that the Sterchi snakes are more sensitive to the presence of humans, and may be stimulated to escape by chemical cues as well as visual cues. The snakes which were not wary of humans may have been selected out by Mr. Sterchi's shotgun. The selection pressure is probably quite high, since many snakes are shot or trapped. Another possibility which seems less likely is that the snakes at Sterchi's tend to be more nocturnal, because of the shooting or because of warmer daily temperatures at Sterchi's. I do not have temperature records, but Tremont is at a slightly higher altitude than Sterchi's, and most of the ground area is shaded.

Food Preference F. It is difficult to draw any support for the previous experiments from this data because newborn snakes from Sterchi's were not obtained, so data from newborns of the two populations cannot be compared. But the data is presented here, and shows a great variability among the responses of litter mates. The highest response is to Desmognathis monticola, a salamander which is common in small creeks and wet areas in the GSMNP. It is possible

that the diets of the snakes vary with age and size, and that young salamanders may be easy to catch for the young snakes. Those with an innate high response for the salamander may have an advantage to help them survive the critical period shortly after birth, and thus may not be competing with the older snakes. There is a higher response to the mountain fish extracts than to the C. auratus extract; this may indicate that the later wide difference has some genetic component. The response to Notropis coccogenis is significantly higher than that to extract of C. auratus. Notropis species are extremely common throughout the range of Natrix sipedon, so this preference may be a genetic advantage.

VIII. DISCUSSION

Related Work on Food Preference

Burghardt (1970) and Fuchs and Burghardt (1971) gave a basis to the technique and some of the aspects of my experiments. The former work showed that naive snakes from geographically different areas had different profiles of prey extract response. The latter showed that the innate profile may be changed by feeding experience in that the response is increased towards extracts of foods eaten. My experiments show that experienced snakes from different ecological areas, with different prey availability, have different prey extract responses.

My techniques differed from theirs in the following ways:

1. Most of their work involved Thamnophis species, and I used Natrix sipedon. This snake is more excitable than Thamnophis, and consequently I increased the trial time from 30 seconds or one minute to two minutes, so that if the snakes became alarmed by the entrance of the swab, they would have time to get calm and respond to it. However, I think that, after examining the attack latency and tongue-flick scores, that one minute would have been sufficient at least for the laboratory snakes. The snakes were alarmed at first, but they seemed to habituate the swab.

2. Burghardt's technique was to hold the swab directly in front of the snake's snout and count all tongue-flicks. I held the swab to one side of the snout and distinguished between tongue-flicks directed toward the swab and "general" tongue-flicks, those not directed toward the swab. For the laboratory snakes, the two measures were not very different and probably the total tongue-flick scores would have shown the same results with the same significant differences as the directed tongue-flick scores. However, in the wild-caught snakes, there were many more "general" tongue-flicks. Some of these were due to unavoidable movements of the experimenter. Often a snake would get very active and seem

to be trying to escape from the testing box. Loop (1970) used a measure of the orienting latency; I found in my snakes that orienting came right before attack.

3. Fuchs and Burghardt used naive or tame experienced snakes, and I used wild-caught snakes which were generally more excitable than tame snakes. One consequence of this was described above. Another consequence was that the snakes experience was unknown to me, but the feeding history could be inferred on this basis of prey availability.

4. Burghardt's (1970) snakes came from widely separated geographical areas, and lived in different drainage areas. The degree of genetic isolation was high, while the degree of ecological variation was unexamined. My two populations of sipedon came from two areas 40 miles apart and in the drainage area of the Tennessee River. The two populations are genetically distant, but not genetically isolated to the degree that Burghardt's snakes were. The important variable between the two populations was the ecological difference. Differences existed in the cover, water conditioned, topography, amount of human disturbance, predator species, and prey species. However, the newborn young from these two areas could conceivably have different prey preferences which are then enhanced by different prey diets and experiences.

Dix (1968) tested the relative preference of experienced garter snakes (Thamnophis s. sirtalis) collected in Massachusetts and Florida. He also tested Natrix s. sipedon from Maryland and Massachusetts and found, in both cases, significant differences. However, he did not establish that the snakes could discriminate between different species of fish or amphibians. His generalized "fish" stimulus was Osmerus mordax, a smelt which is common around the Chesapeake Bay area and lives only in the lower parts of the streams (Eddy, 1969). Since the snakes in Maryland responded more to the smelt stimulus than the Massachusetts snakes did, he concluded that the former had a greater preference for "fish." Had he used fish that were sympatric with the snakes, he probably would have seen a greater response. The response to Rana pipiens was not significantly different for the different populations of snakes. The range of that frog covers the ranges of both species of snakes, and it is probably that the frog shared habitats with the snakes in all localities (Conant, 1958). My experiments show definitely that the snakes can discriminate between different genera of fish. They also indicate that it is the snake's experience in eating the fish that causes the different response to the extract of the fish. Dix's work indicates that snakes from different areas may have

different food preferences; mine goes a step further than that and shows that snakes are likely to have a higher response to prey species with which they are sympatric.

Loop's (1970a) work was useful in setting up my control of H_2O. He showed that rat snakes (Elaphe) could respond to water but did so only when they were water deprived. His 1970b work showed that laboratory-raised Elaphes' experience with a prey (mouse) increased the response to the extract of mouse. This is a similar finding to those of Fuchs and Burghardt, and so further supports my assumption that high responses to extract are due to experiences with the prey.

Further possibilities of this technique

Extract testing could be used to gain more information about ecological relationships of an area. Snakes could be presented with all possible prey species found in an area, and the relative response would indicate what the snakes had been eating. This method would have some advantages over that used by Brown (1940) and others of cutting open the snake to see what its last meal was. Fewer snakes could be used, and each snake could give responses that would indicate its whole feeding pattern, not just the recent one. It would indicate a preference for

prey that is not readily available or is only seasonally available. An example of this is the positive response that Gastrophryne carolinensis extract elicited from the Sterchi snakes. This toad is probably only available during its mating season, which occurs after a heavy rain and lasts about one week. At Sterchi's, I found only one of these toads outside of the mating season, and it was buried in dirt beneath a log. I found no report in the literature that it is eaten by N. sipedon, but the positive response to the extract was confirmed when one of the caged Sterchi snakes ate a specimen. After making extract, I had only one toad left over, so I could not offer any to the Tremont snakes.

Also, some prey might not be detected by the stomach analysis method because of soft bodies (such as worms or amphibian larvae), and the digestion makes the prey items difficult to identify. In addition, stomach analysis does not distinguish between prey ingested by the snake and prey ingested by the snake's prey.

Another advantage of the extract method is that it does not depopulate an area of the study species.

However, there are limitations, and to be useful, extract testing should be used in conjunction with other methods of discovering food habits. A positive extract

response does not prove that a snake eats the extracted prey. The extracts of some prey species may include the same releasing chemicals as others, and some of the chemicals may be similar enough to be generalized. Also, there is no evidence that the relative response to the prey extract is correlated with the relative amount of that prey that is taken. Other variables are the difficulty in catching the prey, the population numbers of the prey, the behavior of the prey, and the role of other sensory cues such as movement, size and form. More experimental work could reduce some of these limitations.

Another limitation to extract testing is that a response to fish cannot be compared to that of an amphibian or to other groups, because, as my observations have shown, the prey catching response of water snakes to fish is different than that to other animals. Sensory modalities are used differently; for example, catching a toad has a greater visual component than catching a fish. Response to extracts involves only the chemical sense modality. So an equal response to toad and minnow cannot be construed to indicate equal preference. Actual food preference tests are necessary.

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